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ALTERED MODULATION OF ADRENERGIC SIGNALING BY SMOOTH MUSCLE CAVEOLAE IN THE OVERACTIVE BLADDER

Hypothesis / aims of study

In bladder smooth muscle (bsm), the role of caveolae, specialized membrane microdomains, in differentially mediating contractile responses to several agonists has been demonstrated. Abnormalities in these signaling microdomains have been implicated in a variety of pathologies. Spontaneously Hypertensive Rats (SHR) exhibit marked spontaneous contractions during bladder filling, and thus provide a relevant animal model of detrusor overactivity (DO)[1]. Along with systemically increased sympathetic activity, a significant decrease in the number of caveolae and alteration in the expression of caveolins (the proteins forming caveolae) have been described in vascular, cardiac, and endothelial tissue of these animals[2]. In this study we investigated whether alterations in membrane caveolae in bsm of SHR contribute to functional abnormalities of adrenergically mediated smooth muscle responses.

Study design, materials and methods

For functional studies, bladder tissue from SHR and the genetic control Wistar-Kyoto rats (WKY) were stretched under 1.5 grams of force and mounted in organ bath maintained at 37ºC. Contraction or relaxation responses to α- and β-adrenoceptor agonists as well as spontaneous bladder activity (SA) were measured after disruption of caveolae by methyl-ß-cyclodextrin (mßcdx) or after caveolae restoration obtained by exposure to soluble cholesterol, or in non-pretreated control tissue. Electron Microscopy (EM) analysis was carried out to detect potential changes in membrane caveolae in SHR and WKY bsm. Quantitative real-time rt-PCR and immunoprecipitation studies were performed to investigate changes respectively in α-and β-adrenoceptor expression and in the interaction between adrenoceptors and caveolin proteins in SHR and WKY.

Results

Amplitude of SA as well as the contractile response to phenylephrine (PE) at baseline levels was significantly higher in SHR compared to WKY. Moreover both SA and the contractile response to PE in WKY were significantly increased after disruption of caveolae compared with SHR in which contractile responses were unaffected by mßcdx. In WKY bladders, cholesterol replenishment restored the amplitude of SA and PE response toward baseline conditions, while SHR bladders were less sensitive to restoration of caveolae with cholesterol. Low concentrations of isoproterenol (ISO) induced a greater dose-dependent bladder relaxation in SHR compared with WKY. However only in SHR, higher concentrations of ISO elicited bladder contractions. These ISO induced contractions in SHR significantly increased after caveolae disruption, but were completely abolished by phenoxybenzamine (POB) pretreatment. After blockade of α-ARs with POB, caveolae disruption attenuated the relaxation response to ISO in both strains, but more so in WKY compared with SHR. Cholesterol replenishment restored relaxation responses toward baseline conditions. EM showed a significant decrease in membrane caveolae in SHR (2.43±1.13 caveolae/□ m) compared with WKY (3.90±1.73 caveolae/□ m). In SHR, both α_{1A}- and α_{1D}-AR were up-regulated in the bladder dome but down-regulated in the body of the bladder compared with WKY. A different interaction between α-AR and caveolins was detected in the two strains: both α_{1A}-AR and α_{1D} -AR co-precipitated only with cav3 in WKY, while in SHR α_{1A} -AR and α_{1D} -AR co-precipitated with both cav3 and cav2. $ß_1$, $ß_2$ and $ß_3$-ARs were down-regulated in the body and unchanged in the dome of SHR compared with WKY; however the relative expression of each ß-subtype was different between the two strains. In SHR, $ß_2$ and $ß_3$ expression was significantly higher than $ß_1$, while in WKY, expression of $ß_3$ and $ß_1$ were greater than $ß_2$.

Interpretation of results

A significant reduction in bladder smooth muscle membrane caveolae in the overactive bladder compared with normal bladders is consistent with the attenuated effect of mβCD and cholesterol on contractile responses in SHR. We demonstrated that a loss of caveolae, either experimentally or biologically generated, resulted in functional alterations in adrenergically-mediated contractile responses, defects in caveolin induced modulation of α- and β-adrenenoceptors and dysregulation of AR-mediated signaling.

Concluding message

Caveolar deficiency results in impaired modulation of adrenergic signaling pathways, suggesting that pathologic aberrations in caveolae and caveolin protein-receptor interactions in bsm cells may lead to the development of DO.

References

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